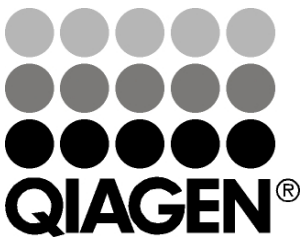
November 2012

GeneRead DNAseq Gene Panel Handbook

For targeted exon enrichment for next-generation sequencing



Sample & Assay Technologies

QIAGEN Sample and Assay Technologies

QIAGEN is the leading provider of innovative sample and assay technologies, enabling the isolation and detection of contents of any biological sample. Our advanced, high-quality products and services ensure success from sample to result.

QIAGEN sets standards in:

- Purification of DNA, RNA, and proteins
- Nucleic acid and protein assays
- microRNA research and RNAi
- Automation of sample and assay technologies

Our mission is to enable you to achieve outstanding success and breakthroughs. For more information, visit www.qiagen.com.

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Kit Contents

GeneRead DNAseq Gene Panel Primer Mixes	**180941***
Tubes with enough primers for 12 or 96 samples, depending on pack size	4
Handbook	1

* Gene panel tubes are labeled A1, B1, C1, and D1.

GeneRead DNAseq Gene Panel High-Content Primer Mixes	**180942†**
Tubes with enough primers for 12 or 96 samples, depending on pack size	8
Handbook	1

† Gene panel tubes are labeled A1, B1, C1, D1, A2, B2, C2, and D2.

GeneRead DNAseq Gene Panel Mix-n-Match Primer Mixes	**180944‡**
Tubes with laboratory-verified primers for 24 samples	4
Handbook	1

‡ Selection limited to 124 genes.

GeneRead DNAseq Gene Panel Custom Primer Mixes	**180946**
Tubes with primers for any gene or genes in the human genome for 500 samples	4
Handbook	1

GeneRead Panel Mastermix*	**(0.6 ml)**	**(4.8 ml)**
Catalog no.	**180962**	**180964**
GeneRead Panel Mastermix, containing: ■ HotStart DNA *Taq* Polymerase ■ PCR Buffer ■ dNTP mix (dATP, dCTP, dGTP, dTTP) DNase-free water	Sufficient reagents for 48 PCR amplification reactions	Sufficient reagents for 384 PCR amplification reactions

Shipping and Storage

GeneRead DNAseq Gene Panel Kits are shipped frozen or at ambient temperature and should be stored at –20°C immediately upon arrival. When stored properly at –20°C, all reagents are stable for up to 6 months after delivery.

GeneRead Panel Mastermixes are shipped on cold packs. For long-term storage, keep tubes at –20°C. If the entire volume will not be used at once, we recommend dividing into aliquots and storing at –20°C. Avoid repeated freezing and thawing. If stored under these conditions, GeneRead Panel Mastermixes are stable for 6 months after receipt.

Product Use Limitations

GeneRead DNAseq Gene Panels and GeneRead Panel Mastermix are intended for molecular biology applications. These products are not intended for the diagnosis, prevention, or treatment of a disease.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

Product Warranty and Satisfaction Guarantee

QIAGEN guarantees the performance of all products in the manner described in our product literature. The purchaser must determine the suitability of the product for its particular use. Should any product fail to perform satisfactorily due to any reason other than misuse, QIAGEN will replace it free of charge or refund the purchase price. We reserve the right to change, alter, or modify any

product to enhance its performance and design. If a QIAGEN product does not meet your expectations, simply call your local Technical Service Department or distributor. We will credit your account or exchange the product — as you wish. Separate conditions apply to QIAGEN scientific instruments, service products, and to products shipped on dry ice. Please inquire for more information.

A copy of QIAGEN terms and conditions can be obtained on request, and is also provided on the back of our invoices. If you have questions about product specifications or performance, please call QIAGEN Technical Services or your local distributor (see back cover or visit www.qiagen.com).

Technical Assistance

At QIAGEN, we pride ourselves on the quality and availability of our technical support. Our Technical Service Departments are staffed by experienced scientists with extensive practical and theoretical expertise in sample and assay technologies and the use of QIAGEN products. If you have any questions or experience any difficulties regarding GeneRead DNAseq Gene Panels or GeneRead Panel Mastermix, or QIAGEN products in general, please do not hesitate to contact us.

QIAGEN customers are a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at QIAGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information, please see our Technical Support Center at www.qiagen.com/Support or call one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit www.qiagen.com).

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at www.qiagen.com/safety where you can find, view, and print the SDS for each QIAGEN kit and kit component.

24-hour emergency information

Emergency medical information in English, French, and German can be obtained 24 hours a day from:

Poison Information Center Mainz, Germany

Tel: +49-6131-19240

Quality Control

In accordance with QIAGEN's Quality Management System, each lot of GeneRead DNAseq Gene Panels and GeneRead Panel Mastermix is tested against predetermined specifications to ensure consistent product quality.

Introduction

DNA resequencing is a useful tool to detect genetic variations, including somatic mutations, SNPs, and small insertions and deletions. Targeted enrichment technology enables next-generation sequencing (NGS) platform users to sequence specific regions of interest instead of the entire genome. GeneRead DNAseq Gene Panels use multiplex PCR-based target enrichment technology in combination with a sophisticated primer design and separation algorithm to enable amplification and enrichment of any gene or targeted region in the human genome in order to detect genetic variation using next-generation sequencing (Figure 1). GeneRead DNAseq Gene Panels are designed to analyze a panel of genes related to a disease state and can be used with any major next-generation sequencing platforms. The targeted enrichment process is essential for the efficient utilization of medium-throughput sequencers such as Life Technologies®' Ion Torrent™ PGM Sequencer and Illumina®'s MiSeq® Personal Sequencer.

GeneRead DNAseq Gene Panels have been optimized in combination with GeneRead Panel Mastermix to provide superior sensitivity and linear multiplex amplification. The simplicity of the PCR method makes these panels accessible for routine use in every research laboratory.

Principle and procedure

GeneRead DNAseq Gene Panels are provided as sets of four tubes, each containing primer mix, with up to 1400 primer pairs. The number of 4-tube sets included is determined by the number of genes in a panel. GeneRead DNAseq Gene Panels can enrich selected genes using as little as 80 ng genomic DNA in two hours (Figure 2). Briefly, add genomic DNA to primer mix and PCR mastermix and put them into a regular thermocycler for PCR amplification. After the reaction is complete, pool the product for the same DNA sample and purify the enriched DNA. The purified DNA then is ready for NGS library construction and sequencing using the NGS platform of your choice. The sequencing results can be analyzed on our server at http://ngsdataanalysis.sabiosciences.com, and genetic variations can be detected (Figure 3).

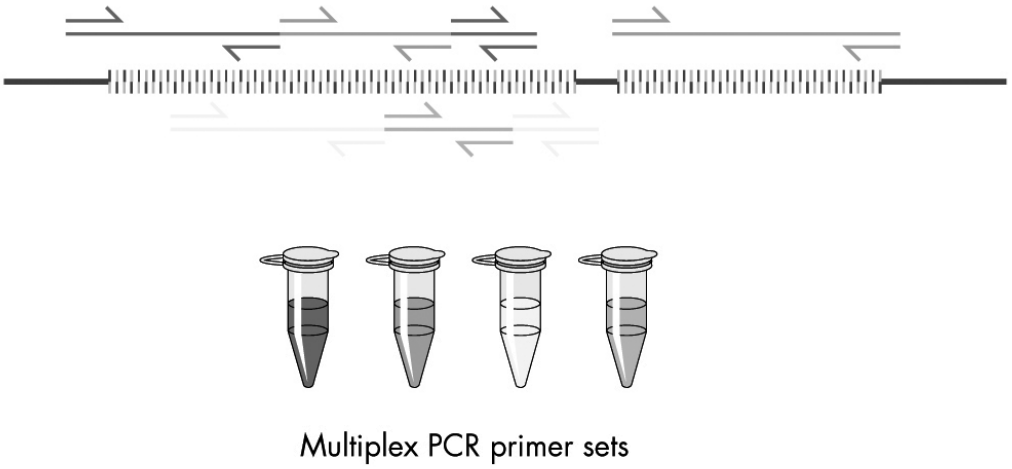

Figure 1. Multiplex PCR-based target enrichment scheme. GeneRead DNAseq Gene Panels use multiplex PCR-based target enrichment technology in combination with a sophisticated primer design and separation algorithm to maximize design coverage and minimize nonspecific amplification.

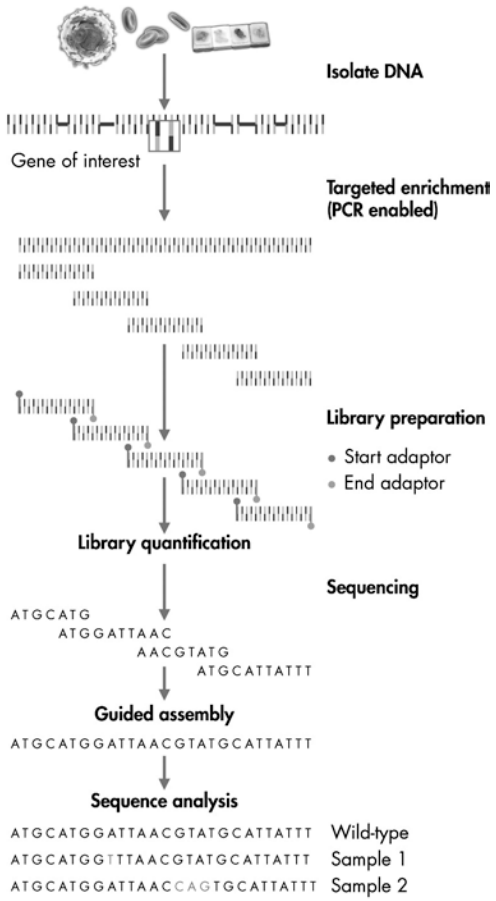

Figure 2. GeneRead DNAseq Gene Panel procedure.

Figure 3. Overview of the NGS workflow with GeneRead DNAseq Gene Panels. The procedure involves DNA extraction (QIAGEN QIAamp DNA Mini Kit or QIAmp DNA FFPE Tissue Kit is recommended), followed by target enrichment with GeneRead DNAseq Gene Panels, NGS library construction, sequencing, and data analysis using the QIAGEN NGS Data Analysis Web Portal.

Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

In addition to the GeneRead DNAseq Gene Panel and GeneRead Panel Mastermix, the following supplies are required:

For genomic DNA isolation:

- See page 13 for specific recommendations.

For target enrichment:

- High-quality, nuclease-free water. **Do not use DEPC-treated water.**
- Agencourt® AMPure® XP Kit
- 70% ethanol
- Low TE
- Magnetic rack for 1.5 ml tubes
- 1.5 ml LoBind tubes
- 0.2 ml PCR tubes, 96-well reaction plates, or PCR strips and caps
- Thermal cycler
- Multichannel pipettor
- Single-channel pipettor
- DNase-free pipet tips and tubes

For NGS library construction for Ion Torrent™ PGM (optional):

- NEBNext Fast DNA Library Prep Set for Ion Torrent
- Agencourt AMPure XP Kit
- 80% ethanol
- QIAGEN's GeneRead DNAseq Library Quant Array for Ion Torrent PGM
- Thermal cycler
- A real-time PCR machine compatible with 96-well plates

For NGS library construction for Illumina MiSeq/HiSeq (optional):

- NEBNext® DNA Library Prep Master Mix Set for Illumina
- NEBNext Multiplex Oligos for Illumina (index primers 1–12, for multiplex sequencing)

- NEBNext Singleplex Oligos for Illumina (for singleplex sequencing)
- Agencourt AMPure XP Kit
- 80% ethanol
- QIAGEN's GeneRead DNAseq Library Quant Array for Illumina
- Thermal cycler
- A real-time PCR machine compatible with 96-well plates

Important Notes

DNA preparation and quality control

High-quality DNA is essential for obtaining good sequencing results

The most important prerequisite for any DNA sequence analysis experiment is consistent, high-quality DNA from every experimental sample. Therefore, sample handling and DNA isolation procedures are critical to the success of the experiment. Residual traces of proteins, salts, or other contaminants will either degrade the DNA or decrease the efficiency of, if not block completely, the enzyme activities necessary for optimal whole genome amplification and real-time PCR performance.

Recommended genomic DNA preparation method

The QIAGEN QIAamp DNA Mini Kit (cat. no. 51304) and QIAamp DNA FFPE Tissue Kit (cat. no. 56404) are highly recommended for the preparation of genomic DNA samples from fresh tissues and FFPE tissue samples. Ensure that samples have been treated for the removal of RNA, as RNA contamination will cause inaccuracies in DNA concentration measurements. **Do not** omit the recommended RNase treatment step to remove RNA. If genomic DNA samples need to be harvested from biological samples for which kits are not available, please contact Technical Support representatives for suggestions.

For best results, all DNA samples should be resuspended in DNase-free water or alternatively in DNase-free 10 mM Tris buffer pH 8.0. **Do not use DEPC-treated water.**

Recommended library quantification method

The QIAGEN GeneRead DNAseq Library Quant Array (cat. no. 180601) is highly recommended for the quantification of the prepared library. Each GeneRead DNAseq Gene Panel contains a set of spike-in controls, and the GeneRead DNAseq Library Quant Array provides predispensed primer assays to measure those controls, for determination of the quality of the prepared sample library. Additionally, the GeneRead DNAseq Library Quant Array contains five predispensed, sequential 10-fold dilutions of Illumina or Ion Torrent DNA Standard mixed with a PCR primer assay in triplicate, and PCR primer assays in the remaining wells of a 96-well, 384-well, or 100-well PCR plate. The predispensed, serially diluted DNA standards and PCR primer assay are a convenient method for quantification of library input. In total, the GeneRead DNAseq Library Quant Array determines quantity as well as quality of the prepared library.

DNA quantification and quality control

For best results, all DNA samples should also demonstrate consistent quality according to the following criteria:

Concentration and purity determined by UV spectrophotometry

The concentration and purity of DNA should be determined by measuring the absorbance in a spectrophotometer. Prepare dilutions and measure absorbance in 10 mM Tris·Cl,* pH 8.0. The spectral properties of nucleic acids are highly dependent on pH.

- A_{260}:A_{230} ratio should be greater than 1.7
- A_{260}:A_{280} ratio should be greater than 1.8
- Concentration determined by A_{260} should be >2.5 µg/ml DNA

DNA integrity

For best results, the genomic DNA should be greater than 2 kb in length with some fragments greater than 10 kb. This can be checked by running a fraction of each DNA sample on a 1% agarose gel.

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

Protocol: PCR Setup

Procedure

1. **Dilute DNA sample to 4 ng/µl. For each sample, 80 ng (20 µl) DNA is required for the 4-pool panel, or 160 ng (40 µl) for the 8-pool panel.**
2. **Determine the number of reactions needed. For a 4-pool panel, 4 reactions for each sample are required. For an 8-pool panel, 8 reactions for each sample are required. Prepare PCR strips or PCR plate according to the number of reactions. Label with sample names and pool numbers.**
3. **Aliquot 5 µl each DNA sample into each well.**
4. **Prepare the PCR reaction mix on ice according to Table 1. For each sample, 4 or 8 PCR reaction mixes will be needed. Mix gently by pipetting up and down.**

Table 1. Preparation of PCR reaction mix for each primer mix pool

Component	Per 1 sample	Per *n* samples
GeneRead Panel Mastermix	11 µl	11 x n µl
Primer mix pool x*	5.5 µl	5.5 x n µl
Total volume	**16.5 µl**	**16.5 x n µl**

* The number of primer mix pools is determined by the panel size.

5. **Aliquot 15 µl of each PCR reaction mix, and put it into the well with DNA samples accordingly. Mix gently by pipetting up and down.**
6. **Seal the wells with PCR tube caps. Place strips or plate in thermocycler and set up reaction parameters according to Table 2.**

Table 2. PCR program

Cycle	Temperature	Time
1	95°C	10 min
20	95°C	15 s
	60°C	2 min
1	72°C	10 min
1	4°C	∞

7. **After the reaction is complete, place the reactions on ice and proceed with sample pooling and purification.**

   Note: If the samples are to be stored prior to purification, transfer them to a –20°C freezer.

Protocol: Sample Pooling and Purification

Procedure

1. **Combine all 4 or 8 reactions from the same sample into one well. Mix thoroughly. The volume of each sample should be approximately 80 µl for a 4-pool panel or 160 µl for an 8-pool panel.**
2. **Transfer 25 µl from each sample to a 1.5 ml Lobind tube for purification. Store the rest at –20°C.**
3. **For each sample, add 45 µl (1.8x volume) of AMPure XP Reagent to the sample and mix by pipetting up and down.**
4. **Incubate for 10 minutes at room temperature.**
5. **Pulse-spin the tube and place in a magnetic rack for 5 minutes or until the beads have collected to the wall of the tube and the solution is clear.**
6. **Carefully remove and discard the supernatant without disturbing the beads.**
7. **Keep the tube on the magnet and add 500 µl freshly prepared 70% ethanol.**
8. **While keeping the tube in the magnetic rack, rotate the tube 180 degrees. When the beads have collected to the opposite side of the tube (near the magnet), turn the tube another 180 degrees. Rotate the tube two more times, each time waiting until the beads collect to the opposite side of the tube.**
9. **Allow the solution to become clear, and carefully remove and discard the supernatant.**
10. **Repeat steps 7–9.**
11. **Pulse-spin the tube, return it to the magnet, and remove any residual ethanol with a pipet.**
12. **Keeping the tube in the magnetic rack, with the cap open, air-dry the beads for 5 minutes at room temperature.**
13. **Resuspend the beads in 25 µl low TE. Mix well by vortexing.**
14. **Pulse-spin the tube, return to the magnet, and collect the supernatant into a new Lobind tube.**
15. **Proceed to library construction according to the sequencing platform of your choice. Refer to Appendix A for recommended library construction protocol for sequencing with Ion Torrent PGM. Refer to**

Appendix B for recommended library construction protocol for sequencing with Illumina MiSeq/HiSeq.

Note: If reactions are to be stored prior to library construction, transfer them to a –20°C freezer.

Troubleshooting Guide

For technical support, please call us at 1-888-503-3187 or 1-301-682-9200. For more information, see also the Frequently Asked Questions page at our Technical Support Center: www.SABiosciences.com/support_faq.php?target=PCR. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocols in this handbook or sample and assay technologies (for contact information, see back cover or visit www.qiagen.com).

References

QIAGEN maintains a large, up-to-date online database of scientific publications utilizing QIAGEN products. Comprehensive search options allow you to find the articles you need, either by a simple keyword search or by specifying the application, research area, title, etc.

For a complete list of references, visit the reference database online at www.SABiosciences.com/support_publication.php#pcrarray or contact QIAGEN Technical Services or your local distributor.

Appendix A: Library Construction using the NEBNext Fast DNA Library Prep Set for Ion Torrent

Procedure

End repair of DNA

A1. Add the components in Table 3 to a 0.2 ml PCR tube on ice.

Table 3. DNA end-repair reaction components

Component	Volume
PCR-enriched DNA from previous step	25 µl
NEBNext End Repair Reaction Buffer	6 µl
NEBNext Repair Enzyme Mix	3 µl
DNase-free water	26 µl
Total	**60 µl**

A2. Mix the components by pipetting up and down several times.

A3. Incubate in a thermal cycler for 20 minutes at 25°C, followed by 10 minutes at 70°C.

A4. Pulse-spin the microfuge tube and return to ice.

Preparation of adaptor-ligated DNA

A5. Add the reagents in Table 4 to the PCR tube.

Table 4. Reagents for preparation of adaptor-ligated DNA

Component	Volume
DNase-free water	14 µl
T4 DNA Ligase Buffer (10x)	10 µl
NEBNext DNA Library Adaptors for Ion Torrent	10 µl
T4 DNA Ligase	6 µl
Total	**40 µl**

A6. The total volume in the microfuge tube should be 100 µl. Mix the contents by pipetting up and down several times.

A7. Incubate in a thermal cycler for 15 minutes at 16°C.

A8. Transfer the entire volume to a 1.5 ml Lobind tube.

Cleanup of adaptor-ligated DNA

A1. Add 160 µl (1.6x volume) AMPure XP Reagent to the sample and mix by pipetting up and down.

A2. Incubate for 5 minutes at room temperature.

A3. Pulse-spin the tube and place in a magnetic rack for 5 minutes or until the beads have collected to the wall of the tube and the solution is clear.

A4. Carefully remove and discard the supernatant without disturbing the beads.

A5. Keep the tube on the magnet and add 400 µl freshly prepared 80% ethanol.

A6. While keeping the tube in the magnetic rack, rotate the tube 180 degrees. When the beads have collected to the opposite side of the tube (near the magnet), turn the tube another 180 degrees. Rotate the tube two more times, each time waiting until the beads collect to the opposite side of the tube.

A7. Allow the solution to become clear, and carefully remove and discard the supernatant.

A8. Repeat previous three steps (A5–A7).

A9. Pulse-spin the tube, return to the magnet, and remove any residual ethanol with a pipet.

A10. Keeping the tube in the magnetic rack, with the cap open, air-dry the beads for 5 minutes at room temperature.

A11. Resuspend the beads in 150 µl sterile water. Mix well by vortexing.

A12. Pulse-spin the tube, return to the magnet, and collect the supernatant.

Size selection

A1. Add 105 µl (0.7x) AMPure XP beads to 150 µl DNA solution. Mix well on a vortex mixer or by pipetting up and down at least 10 times.

A2. Incubate for 5 minutes at room temperature.

A3. Pulse-spin the tube and place tube on magnetic rack to separate beads from supernatant. After the solution is clear (about 5 minutes), carefully transfer the supernatant to a new tube. Discard the beads which contain the large fragments.

Note: Do not the discard the supernatant!

A4. Add 120 µl (0.8x) AMPure XP beads to the supernatant, mix well and incubate for 5 minutes at room temperature.

A5. Pulse-spin the tube and place tube on magnetic rack and wait until solution is clear (about 5 minutes). Carefully remove and discard supernatant. Be careful not to disturb the beads, which contain the DNA target.

Note: Do not discard beads.

A6. Add 400 µl fresh 80% ethanol to the tube while on magnetic rack. Incubate at room temperature for 30 seconds, and then carefully remove and discard the supernatant.

A7. Repeat step A6 once.

A8. Briefly spin the tube, and place on magnetic rack. Completely remove residual ethanol and dry beads for 10 minutes while tube is on rack with lid open.

A9. Elute DNA target beads into 25 µl 0.1x TE buffer. Mix well by vortexing. Spin down briefly and place tube on rack until solution is clear.

A10. Transfer the supernatant to a clean PCR tube and proceed to PCR amplification.

PCR amplification of adaptor-ligated DNA

A1. Mix the components in Table 5 in a 0.2 ml PCR tube.

Table 5. Reaction components for PCR amplification

Component	Volume
Adaptor-ligated DNA	25 µl
Primers	4 µl
DNase-free water	21 µl
OneTaq® Hot Start 2x Master Mix	50 µl
Total	**100 µl**

A2. Set up the cycler using the cycling conditions in Table 6.

Table 6. Cycling conditions for amplification of adaptor-ligated DNA

Step	Temperature	Time
Nick translation	68°C	20 min
Initial denaturation	94°C	30 sec
5 cycles	94°C	30 sec
	58°C	30 sec
	68°C	1 min
Hold	4°C	∞

A3. Transfer the entire volume to a 1.5 ml Lobind tube.

Cleanup of adaptor-ligated DNA

A1. Add 140 µl (1.4X volume) of AMPure XP Reagent to the sample and mix by pipetting up and down.

A2. Incubate for 5 minutes at room temperature.

A3. Pulse-spin the tube and place in a magnetic rack for 5 minutes or until the beads have collected to the wall of the tube and the solution is clear.

A4. Carefully remove and discard the supernatant without disturbing the beads.

A5. Keep the tube on the magnet and add 400 µl freshly prepared 80% ethanol.

A6. While keeping the tube in the magnetic rack, rotate the tube 180 degrees. When the beads have collected to the opposite side of the tube (near the magnet), turn the tube another 180 degrees. Rotate the tube two more times, each time waiting until the beads collect to the opposite side of the tube.

A7. Allow the solution to become clear, and carefully remove and discard the supernatant.

A8. Repeat previous three steps (A5–A7).

A9. Pulse-spin the tube, return to the magnet, and remove any residual ethanol with a pipet.

A10. Keeping the tube in the magnetic rack, with the cap open, air-dry the beads for 5 minutes at room temperature.

A11. Resuspend the beads in 22 µl 0.1x TE buffer. Mix well by vortexing.

A12. Pulse-spin the tube, return to the magnet, and collect the supernatant.

Library quantification using GeneRead DNAseq Library Quant Array

The library can be stored in a –20°C freezer prior to quantification.

Appendix B: NEBNext DNA Library Prep Master Mix Set for Illumina with NEBNext Singleplex Oligos for Illumina (E7350) or NEBNext Multiplex Oligos for Illumina (Index Primers 1–12) (E7335)

Procedure

Adaptor ligation of PCR product

B1. Add the components from Table 7 to a 0.2 ml PCR tube.

Table 7. Reagents for adaptor ligation of PCR product

Component	Volume
Purified PCR product	25 µl
Quick Ligation Reaction Buffer (5X)	10 µl
NEBNext Adaptor	2 µl
Quick T4 DNA Ligase	2 µl
DNase-free water	11 µl
Total	**50 µl**

B2. Incubate in a thermal cycler for 15 minutes at 20°C.

B3. Add 3 µl USER™ enzyme mix by pipetting up and down and incubate at 37°C for 15 minutes.

B4. Transfer the entire volume to a 1.5 ml Lobind tube.

Cleanup using AMPure XP Beads

B1. Add 90 µl (1.8X volume) of AMPure XP Reagent to the sample and mix by pipetting up and down.

B2. Incubate for 5 minutes at room temperature.

B3. Pulse-spin the tube and place in a magnetic rack for 5 minutes or until the beads have collected to the wall of the tube and the solution is clear.

B4. Carefully remove and discard the supernatant without disturbing the beads.

B5. Keep the tube on the magnet and add 400 µl freshly prepared 80% ethanol.

B6. While keeping the tube in the magnetic rack, rotate the tube 180 degrees. When the beads have collected to the opposite side of the tube (near the magnet), turn the tube another 180 degrees. Rotate the tube two more times, each time waiting until the beads collect to the opposite side of the tube.

B7. Allow the solution to become clear, and carefully remove and discard the supernatant.

B8. Repeat previous three steps (B5–B7).

B9. Pulse-spin the tube, return to the magnet, and remove any residual ethanol with a pipet.

B10. Keeping the tube in the magnetic rack, with the cap open, air dry the beads for 5 minutes at room temperature.

B11. Resuspend the beads in 150 µl sterile water. Mix well by vortexing.

B12. Pulse-spin the tube, return to the magnet, and collect the supernatant.

Size selection

B1. Add 120 µl (0.8x) AMPure XP beads to 150 µl DNA solution. Mix well on a vortex mixer or by pipetting up and down at least 10 times.

B2. Incubate for 5 minutes at room temperature.

B3. Pulse-spin the tube and place tube on magnetic rack to separate beads from supernatant. After the solution is clear (about 5 minutes), carefully transfer the supernatant to a new tube. Discard the beads which contain the large fragments.

Note: Do not the discard the supernatant.

B4. Add 90 µl (0.6x) AMPure XP beads to the supernatant, mix well and incubate for 5 minutes at room temperature.

B5. Pulse-spin the tube and place tube on magnetic rack and wait until solution is clear (about 5 minutes). Carefully remove and discard supernatant. Be careful not to disturb the beads which contain the DNA target.

Note: Do not discard the beads.

B6. Add 400 µl fresh 80% ethanol to the tube while on magnetic rack. Incubate at room temperature for 30 seconds, and then carefully remove and discard the supernatant.

B7. Repeat step B6 once.

B8. Briefly spin the tube, and place on magnetic rack. Completely remove residual ethanol and dry beads for 10 minutes while tube is on rack with lid open.

B9. Elute DNA target beads into 23 µl 0.1x TE buffer. Mix well by vortexing. Spin down briefly and place tube on rack until solution is clear.

B10. Transfer the supernatant to a clean PCR tube and proceed to PCR amplification.

PCR amplification of adaptor-ligated DNA

B1. Add the reagents in Table 8 to a 0.2 ml PCR tube.

Table 8. Reagents for PCR amplification of adaptor-ligated DNA

Component	Volume
Adaptor-ligated DNA	23 µl
Universal PCR primer (25 µM)	1 µl
Index primer (1)* (25 µM)	1 µl
Phusion® High-Fidelity PCR Master Mix with HF Buffer, 2X	25 µl
Total	**50 µl**

* If NEBNext Multiplex Oligos for Illumina (Index Primers 1-12) are used, for each reaction, only one of the 12 PCR primer indices is used during the PCR step.

B2. Set up the cycler using the cycling conditions in Table 9.

Table 9. Cycling conditions for PCR amplification of adaptor-ligated DNA

Step	Temperature	Time
Initial denaturation	98°C	30 sec
12 cycles	98°C	10 sec
	65°C	30 sec
	72°C	30 sec
Index primer (1)* (25 µM)	98°C	5 min
Phusion High-Fidelity PCR Master Mix with HF Buffer, 2X	98°C	∞

B3. Transfer the entire volume to a 1.5 ml Lobind tube.

Cleanup using AMPure XP Beads

B1. Add 60 µl (1.2x volume) AMPure XP Reagent to the sample and mix by pipetting up and down.

B2. Incubate for 5 minutes at room temperature.

B3. Pulse-spin the tube and place in a magnetic rack for 5 minutes or until the beads have collected to the wall of the tube and the solution is clear.

B4. Carefully remove and discard the supernatant without disturbing the beads.

B5. Keep the tube on the magnet and add 400 µl freshly prepared 80% ethanol.

B6. While keeping the tube in the magnetic rack, rotate the tube 180 degrees. When the beads have collected to the opposite side of the tube (near the magnet), turn the tube another 180 degrees. Rotate the tube two more times, each time waiting until the beads collect to the opposite side of the tube.

B7. Allow the solution to become clear, and carefully remove and discard the supernatant.

B8. Repeat previous three steps (B5–B7).

B9. Pulse-spin the tube, return to the magnet, and remove any residual ethanol with a pipet.

B10. Keeping the tube in the magnetic rack, with the cap open, air-dry the beads for 5 minutes at room temperature.

B11. Resuspend the beads in 22 µl 0.1x TE Buffer. Mix well on a vortex mixer or by pipetting up and down, and put the tube in the magnetic stand until the solution is clear.

B12. Transfer supernatant to a clean 1.5 ml LoBind tube.

Library quantification using GeneRead DNAseq Library Quant Array

The library may be stored in a –20°C freezer prior to quantification.

Appendix C: Library Quantification and Quality Control

Quality control for the target enrichment and library construction process can be performed using QIAGEN's GeneRead DNAseq Library Quant Array. With this array, the correct dilution of the library can also be determined for sequencing. Please refer to the corresponding user manual for library quantification and QC.

Appendix D: Data Analysis using QIAGEN Web Portal

After sequencing, results can be analyzed using QIAGEN's Next-Generation Sequencing Data Analysis Web Portal. Our data analysis server will perform reads trimming (removing primer sequences), mapping to reference genome, and variants identification. Please refer to the corresponding document for data analysis.

Ordering Information

Product	Contents	Cat. no.
GeneRead DNAseq Gene Panels	Sets of 4 tubes containing wet-bench verified primer sets for targeted exon enrichment of a pathway-focused panel of genes	180941
GeneRead DNAseq Gene Panels: High-Content	Sets of 8 tubes containing wet-bench verified primer sets for exon enrichment of a pathway-focused panel of genes	180942
GeneRead Custom DNAseq Gene Panels	Tubes containing primer sets for targeted exon enrichment of a customized panel of genes	180946
GeneRead DNAseq Mix'n'Match Gene Panels	Tubes containing wet-bench verified primer sets for targeted exon enrichment of a custom panel of genes	180944
GeneRead Panel Mastermix	Mastermix for use with the GeneRead DNAseq Gene Panel System	Varies
Related products		
GeneRead DNAseq Library Quant Array	Reagents for NGS sample library quantification following targeted exon enrichment with the GeneRead DNAseq Gene Panel System	180601
GeneRead Library Quant Array	Reagents for NGS sample library quantification	180611
GeneRead Library Quant Kit	Reagents for NGS sample library quantification	180612
GeneRead qPCR SYBR Green Mastermix	Mastermix for use with the GeneRead Library Quant Arrays and Kit	Varies
QIAamp DNA Mini Kit (50)	For 50 DNA preps: 50 QIAamp Mini Spin Columns, QIAGEN Proteinase K, Collection Tubes (2 ml), reagents and buffers	51304

Product	Contents	Cat. no.
QIAamp DNA FFPE Tissue Kit (50)	For 50 DNA preps: 50 QIAamp MinElute Columns, Proteinase K, Collection Tubes (2 ml), buffers	56404

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